Sequencing of bulked BAC clones on chromosome 3H of barley physical and genetic maps

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Haruna Nijo BAC library was applied for clone selection by primer sets derived from genetically and physically mapped ESTs on chromosome 3H and the pooled barley BAC clones were sequenced by 454 parallel genome sequencer.

For the selection of genetically mapped ESTs, a high-resolution transcript linkage map of barley was created using a single doubled haploid mapping population, only 3’-end ESTs, and only PCR-based assays (Fig. 1). Cultivar “Haruna Nijo” and an ancestral wild form accession “H602” were used as EST donors and crossing parents of the mapping population. Of the 2,890 ESTs mapped by SNPs (1,717), CAPS (933) and INDELs (240), 444 ESTs were mapped on chromosome 3H.

For the selection of physically mapped ESTs, we used the gametocidal system inducing chromosomal structural changes in the 3H addition line of common wheat, and we cytologically screened for rearranged chromosomes involving the 3H chromosome by in situ hybridization (FISH/GISH) (Fig. 2). We used these dissection lines to map 36 EST markers that were polymorphic between euploid common wheat and the 3H addition line and that had been used for the construction of a 3H genetic map. The results of the PCR analyses placed the 36 EST markers into 20 chromosomal regions flanked by the breakpoints of the dissected chromosomes. These markers were used for BAC clone selection (Fig. 3).

The DNA samples of 10 or 20 BAC clones were pooled and used for shotgun library development. Contig sequences generated in each pooled library were compared homology with mapped EST sequences. Their lengths ranged 1,230 bp to 58,322 bp with an average 14,891 bp (Fig. 4). Of these contigs, 255 showed homology and colinearity with the genome sequence of rice chromosome 1. A contig annotation browser functioned with query search by unique sequence or genetic map position was developed (Fig. 5). The identified contig can be annotated with barley cDNAs and reference sequences on the browser. Homology analysis of these contigs with rice genes indicated that 2,959 rice genes can be involved in barley contigs by the simple comparison of sequence lengths in both species. Of these genes, 753 are assigned to rice chromosome 1. Thus, an efficient sequencing strategy on gene rich region in barley chromosome 3H with special reference to rice chromosome 1 was demonstrated.